

PRINTER RUSH
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Application : 09/936,759 Examiner : Vogel GAU : 1636

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[RUSH] MESSAGE: please provide missing U.S. Patent No.
on page 29 line #5 of specification!

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814 32nd Ave S
Seattle WA 98144 U.S.A.
+1 206 860 2120
+1 206 860 3933 fax

Cougar Patent Law

Fax

To:	Thomas Hawkins	From:	Carol Nottenburg
Fax:	1 571 273 9773	Pages:	2
Phone:	1 703 308 9250	Date:	02/28/06
Re:	09/936,759	Ref:	CAMBIA 40501US

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tetracycline resistance, kanamycin resistance, chloramphenicol resistance, and the like. The selectable marker also preferably has a linked constitutive or inducible promoter and a termination sequence, including a polyadenylation signal sequence. Other selection systems, such as positive selection can alternatively be used (U.S. Patent
 5 Nos. US 5994629).

The sequence of nucleotides encoding β -glucuronidase may also include a classical secretion signal, whereby the resulting peptide is a precursor protein processed and secreted. Suitable signal sequences of plant genes include, but are not limited to the signal sequences from glycine-rich protein and extensin. In addition, a
 10 glucuronide permease gene to facilitate uptake of glucuronides may be co-transfected either from the same vector containing microbial GUS or from a separate expression vector.

A general vector suitable for use in the present invention is based on pBI121 (U.S. Patent No. 5,432,081) a derivative of pBIN19. Other vectors have been
 15 described (U.S. Patent Nos. 4,536,475; 5,733,744; 4,940,838; 5,464,763; 5,501,967; 5,731,179) or may be constructed based on the guidelines presented herein. The plasmid pBI121 contains a left and right border sequence for integration into a plant host chromosome and also contains a bacterial origin of replication and selectable marker. These border sequences flank two genes. One is a kanamycin resistance gene
 20 (neomycin phosphotransferase) driven by a nopaline synthase promoter and using a nopaline synthase polyadenylation site. The second is the *E. coli* GUS gene (reporter gene) under control of the CaMV 35S promoter and polyadenylated using a nopaline synthase polyadenylation site. The *E. coli* GUS gene is replaced with a gene encoding a secreted form of β -glucuronidase. If appropriate, the CaMV 35S promoter is replaced
 25 by a different promoter. Either one of the expression units described above is additionally inserted or is inserted in place of the CaMV promoter and GUS gene.

Plants may be transformed by any of several methods. For example, plasmid DNA may be introduced by *Agrobacterium* co-cultivation (e.g., U.S. Patent No. 5,591,616; 4,940,838) or bombardment (e.g., U.S. Patent No. 4,945,050; 5,036,006;
 30 5,100,792; 5,371,015). Other transformation methods include electroporation (U.S.